Improving the thermal stability of Rubisco activase

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Abstract

The thermal sensitivity of the key photosynthetic enzyme Rubisco activase limits wheat photosynthesis at moderately high temperatures. Introduction of a more thermal tolerant form of Rubisco activase, such as that from cotton, into wheat is predicted to broaden the temperature range of optimal Rubisco activation and photosynthetic CO_2 assimilation. Transgenic lines have been produced to express the cotton Rubisco activase in wheat and current efforts are characterizing the most promising lines for further studies of photosynthetic performance at moderately high temperatures. Joanna Scales, a BBSRC PhD student jointly supervised by Martin Parry, Christine Raines, and Mike Salvucci, generated the transformation constructs and will undertake the molecular and biochemical analysis of the transformant lines. It is predicted that the cotton Rubisco activase will confer superior thermal tolerance to wheat photosynthesis.

Introduction

The primary determinant of crop biomass is cumulative photosynthesis over the growing season. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme; it catalyzes the assimilation of CO_2 through carboxylation of RuBP in the Calvin-Benson cycle. Rubisco is prone to inactivation by non-productive binding of sugar-phosphates to active sites (Parry et al. 2013). Conformational remodeling by the specific chaperone, Rubisco activase, is required to reactivate Rubisco. Rubisco activase is extremely sensitive to elevated temperatures and an important determinant of the heat stability of photosynthesis (Salvucci and Crafts-Brandner 2004; Barta et al. 2010). Importantly, the Rubisco activase from cool-season species, such as wheat, becomes inactive at considerably lower temperatures than the enzyme from warm-adapted species, such as cotton (Carmo-Silva and Salvucci 2011). The goal of this project is to produce wheat germplasm with improved photosynthetic tolerance to heat stress by introducing cotton Rubisco activase into wheat.

Materials and methods

Constructs were made to express cotton Rubisco activase α- and β-isoforms (Salvucci et al. 2003) in wheat, using a vector designed at Rothamsted Research (Dr. Alison Huttly). The two isoforms are being expressed independently because of their different regulatory properties (Carmo-Silva and Salvucci 2013). Biolistic transformation of wheat (*Triticum aestivum* cv. Cadenza) was carried out by the Cereal Transformation Group at Rothamsted Research to generate transgenic plants expressing the cotton Rubisco activase (Sparks and Jones 2014).

The activation of Rubisco purified from wheat by recombinant cotton Rubisco activase was measured in vitro as previously described (Barta et al. 2010). The presence of the cotton Rubisco activase transgene is being verified by PCR analyses. Rubisco activase gene expression levels and protein amounts in plants harboring the foreign DNA are being determined by RT-qPCR and Western Blotting. Further molecular, biochemical, and physiological studies will include characterization of the amounts of cotton and wheat Rubisco activase isoforms in the transgenic lines and the corresponding effects on Rubisco activation and photosynthesis at different temperatures (Carmo-Silva and Salvucci 2012; Scales et al. 2014).

Results

In vitro experiments confirmed that recombinant cotton Rubisco activase competently activates Rubisco purified from wheat. Hence, the duo cotton Rubisco activase and wheat Rubisco is expected to function in vivo. Constructs

were made to target the cotton Rubisco activase to the wheat chloroplast, with a constitutive promoter plus the cotton genes for either the α-isoform or the β-isoform of activase, including their respective transit peptide. To increase the probability of success, an additional pair of constructs was made using the wheat Rubisco small subunit transit peptide. A number of transformed wheat lines have been identified for each of the four constructs (Table 1) and T_0 plants of the most recent lines or T_1 plants of the most advanced lines are being grown in the glasshouse (Fig. 1).

Table 1. Transformant lines of wheat produced on a Cadenza background for expression of the cotton Rubisco activase (Rca) α- and β-isoforms using the corresponding cotton Rca transit peptide or the wheat Rubisco small subunit (rbcS) transit peptide to target the protein to the chloroplast. The production of these lines was funded by CIMMYT and the 20:20 Wheat® ISP of Rothamsted Research.

Gene of interest	Transit peptide	Transformant lines
Cotton *Rca* α-isoform	Cotton *Rca*	17
Cotton *Rca* β-isoform	Cotton *Rca*	26
Cotton *Rca* α-isoform	Wheat *rbc*S	25
Cotton *Rca* β-isoform	Wheat *rbc*S	25

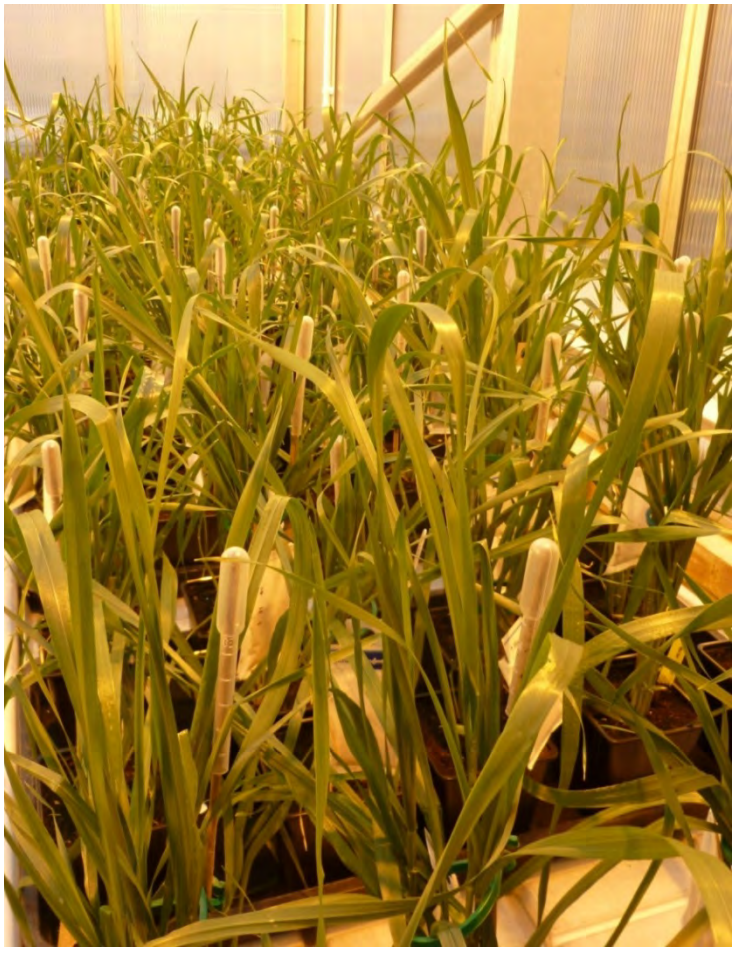

Figure 1. Plants of wheat lines transformed with the cotton Rubisco activase genes, growing in the glasshouse.

In order to identify wheat transformant lines expressing significant amounts of cotton Rubisco activase, a preliminary experiment was carried out to characterize the separation of wheat and cotton α- and β-isoforms of Rubisco activase (Fig. 2). In wheat the α-isoform of Rubisco activase is present in very low amounts compared to the β-isoform. Contrarily, in cotton, the α-isoform is present in only marginally lower amounts than the β-isoform. Importantly, the isoforms from wheat and cotton are very close in molecular weight and could not be readily separated under the conditions used for gel electrophoresis.

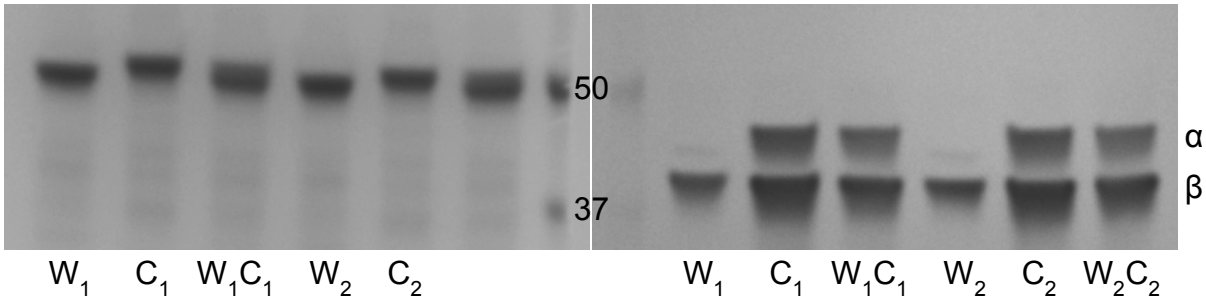

Figure 2. Rubisco (left hand-side image, SDS-Page) and Rubisco activase α- and β-isoforms (right hand-side image, immunoblotting) in leaf extracts of wild-type wheat (W), cotton (C) or a mix of both in equal amounts (WC). Molecular weight markers of 50 and 37 kDa are indicated for reference. Proteins were extracted from duplicate samples (1 and 2) of young fully-expanded leaves of wild-type cotton (*Gossypium hirsutum* cv. Vicky) and wheat (*Triticum aestivum* cv. Cadenza). An identical amount of 2 µg total soluble protein was loaded of each sample into an 8-16% precise protein gel (15 well; Thermo Scientific). After separation of polypeptides by SDS-Page, half of the gel was stained with Coomassie Blue and the other half was immunoblotted with antibody against Rubisco activase (Carmo-Silva and Salvucci 2012).

Discussion and conclusions

Rubisco activation decreases at moderately high temperatures, compromising photosynthetic capacity and efficiency. Wheat transgenic lines have been produced to express the more thermally stable Rubisco activase from cotton. The hypothesis is that wheat plants expressing both the cool-adapted wheat and the warm-adapted cotton Rubisco activase isoforms will operate at a broader range of temperatures (Carmo-Silva and Salvucci 2011). Transformant plants of T_0 and T_1 generations are now being characterized for the presence of the transgene, expression of Rubisco activase and protein amounts.

The wheat and cotton Rubisco activase isoforms were not readily distinguished by SDS-Page. A possible alternative to distinguish between the wheat and cotton Rubisco activase isoforms is two-dimensional electrophoresis, using isoelectric-focusing followed by SDS-Page. Nonetheless, if the cotton Rubisco activase is expressed in considerable amounts and if the expression of the native wheat Rubisco activase remains unaffected, the increase in amount of protein will be observed using SDS-Page only. Analysis of gene expression by RT-qPCR will provide further evidence of wheat lines with significant amounts of cotton Rubisco activase. These lines will be taken through the T_3 generation and plants will then be fully characterized for their temperature response of Rubisco activation and photosynthesis. Lines with improved thermal tolerance of photosynthesis will be tested under controlled environments and then field conditions.

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